RELATION OF ANTIBODY AND ANTIGEN TO SERUM DISEASE SUSCEPTIBILITY.

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Longcope and Rackemann[1] have reported observations on the precipitin, anaphylactic antibody, and cutaneous hypersensitiveness in individuals to whom serum had been administered therapeutically. In the course of these studies, two patients were encountered who developed no serum disease. It was found that in the blood of these patients, no precipitin was demonstrable. The studies reported in the present paper were undertaken with a threefold purpose: (1) to pursue further the investigation of the relations between precipitin formation and the symptoms of serum reactions; (2) to determine whether or not the disappearance of horse serum from the circulation can be brought into relation with precipitin formation or the symptoms; and (3) to investigate further the factors concerned in the non-susceptibility of certain individuals to serum sickness.

Method.

The presence in the patients' serum of the antipneumococcus or antimeningococcus horse serum, which we refer to as precipitinogen, was determined by specific precipitation with the serum of rabbits immunized against horse serum. This anti-horse rabbit serum was used in all the tests without inactivation, and without preservatives, but was diluted with an equal volume of normal salt solution. On each day on which tests were done, the rabbit serum was titrated

* The participation of Dr. Leake in the investigation reported in this paper was made possible by the donation of a fund by Mrs. Arthur W. Swan.

[1] Longcope, W. T., and Rackemann, F. M., *J. Exp. Med.*, 1918, xxvii, 341.

against dilutions of normal horse serum, and to insure the specificity of the reactions, it was titrated against several dilutions of normal human serum. Owing to the frequent occurrence of prozone phenomena, it was found necessary to set up several dilutions of each of these controls, because sometimes an anti-horse rabbit serum which gives no precipitation with human serum in dilutions of 1:2 or 1:10 will give a definite clouding or actual precipitate in dilutions of 1:100 or 1:1,000. Obviously, any such undetected non-specific reactions would lead to false conclusions as to the presence of precipitinogen in the patients' serum.

In the determination of precipitinogen, there is another possible source of error which must be guarded against. It is, of course, well known that an immune serum may contain both precipitin and precipitinogen without forming a precipitate.[2, 3] But if two such sera are mixed, precipitation follows. In determining the titer, therefore, of the precipitinogen in the patient's serum, it is important to use only immune sera which have been tested and found to be free from precipitinogen. Otherwise when precipitin is present in the serum of the patient, it will precipitate these persisting traces of precipitinogen in the immune rabbit serum and make it appear that precipitinogen is still present in the circulation of the patient. The absence of precipitinogen from the immune rabbit serum is demonstrated by securing two anti-horse rabbit sera which, when titrated against each other, produce no precipitation.

Determinations of the precipitin in the patient's serum were made by the usual method of setting up the patient's serum, diluted with an equal volume of salt solution, against several dilutions of horse serum. In all the tests, both for precipitin and precipitinogen, a constant amount of the precipitating serum was added to increasing dilutions of precipitinogen.

Usually the tests were done on the same day that the blood was drawn, but in a few instances the serum was kept in the ice box over night before being used. After being set up, the tubes were placed in the water bath for 1 hour at 37°C. and then in the ice box over night, when the final reading was made.

[2] Weil, R., *Proc. Soc. Exp. Biol. and Med.*, 1914–15, xii, 37.

[3] Denzer, B. S., *J. Infect. Dis.*, 1916, xviii, 631.

The delicacy of these tests is considerably increased if the serum is perfectly clear. For several hours after an ordinary meal, the serum is so clouded with fat that accurate readings are impossible. Whenever possible, therefore, the blood was drawn after a 14 hour fast. This almost always insures a perfectly clear serum.

Material.

By the methods described above, we have studied twenty-one patients to whom serum was administered for therapeutic purposes either intravenously or intraspinally in amounts varying from 34 to 630 cc. There were, however, two patients in this group who, we felt, should be excluded from analysis because of complicating factors, affecting to an unknown degree the phenomena under consideration. One of these was a patient with lobar pneumonia, Type I, syphilis, and a severe amebic colitis. As he was having frequent watery stools, it seemed probable that he might be eliminating the foreign serum through the intestinal mucosa. If such were the case, the precipitinogen would disappear from the circulation earlier than it would if only the usual mechanism for elimination were operating. The same consideration holds for the other patient excluded from analysis. This patient was a child 5 years old with Pneumococcus Type I peritonitis and empyema. Laparotomy and thoracotomy were done and the abdomen and pleural cavity were drained. It was therefore thought to be highly probable that she was eliminating precipitinogen through the abdominal and thoracic sinuses and that conditions were not comparable to those existing in the other patients. It is noteworthy that the discrepancy observed in these two patients in the interrelations of precipitin, precipitinogen, and symptoms was merely the unusually early disappearance of precipitinogen from the circulation.

The nineteen patients remaining for consideration varied in age from 13 to 56. There were thirteen males and six females. They were treated with antipneumococcus or antimeningococcus serum in amounts varying from 34 to 630 cc. Eighteen were patients with lobar pneumonia, Type I, and one was a patient with meningococcus meningitis. Only one of the patients showed cutaneous hypersen-

sitiveness prior to the therapeutic administration of serum. The single individual who was hypersensitive when he came under observation will be referred to again. These data regarding the patients studied are shown in Table I.

TABLE I.

Patient No.	Age.	Sex.	Diagnosis.	Amount of serum.	Method of administration.	Intracutaneous test with horse serum before administration of serum.
	yrs.			*cc.*		
1	56	M.	Lobar pneumonia.	100	Intravenously.	Negative.
2	20	"	" "	200	"	"
3	39	F.	Meningococcus meningitis.	95	Intraspinally.	"
4	25	M.	Lobar pneumonia.	500	Intravenously.	"
5	25	F.	" "	300	"	"
6	24	"	" "	320	"	"
7	32	M.	" "	480	"	"
8	20	"	" "	300	"	"
9	37	"	" "	630	"	"
10	25	"	" "	370	"	"
11	32	F.	" "	270	"	"
12	22	M.	" "	34	Subcutaneously, 2 cc. Intravenously, 32 "	Positive.
13	23	F.	" "	500	"	Negative.
14	13	M.	" "	180	"	"
15	25	"	" "	380	"	"
16	19	"	" "	385	"	"
17	28	"	" "	520	"	"
18	26	"	" "	200	"	"
19	32	F.	" "	500	"	"

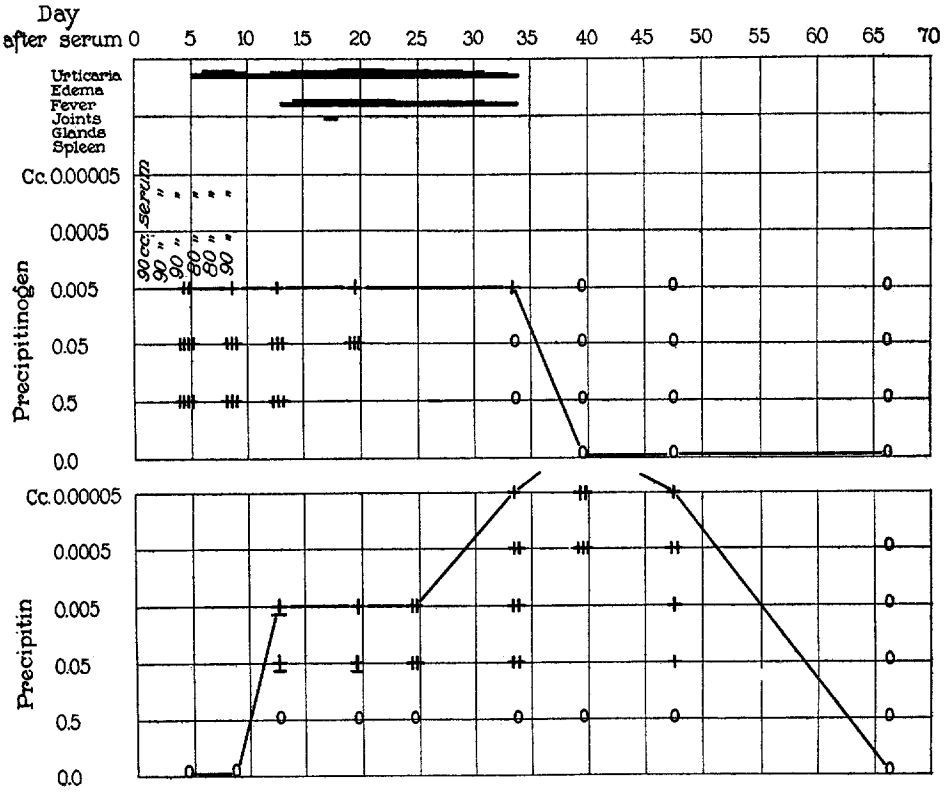
RESULTS.

Analysis of the results obtained in this series of nineteen patients shows that from the point of view of circulating precipitin, persistence of precipitinogen, and severity of symptoms, they may be divided into three groups.

Group 1.—Eleven of the nineteen patients fall quite sharply into one group. This group has the following characteristics: (1) Severe serum disease. If the onset of the serum reaction is considered to be marked by the appearance of an eruption, edema, or arthralgia, and

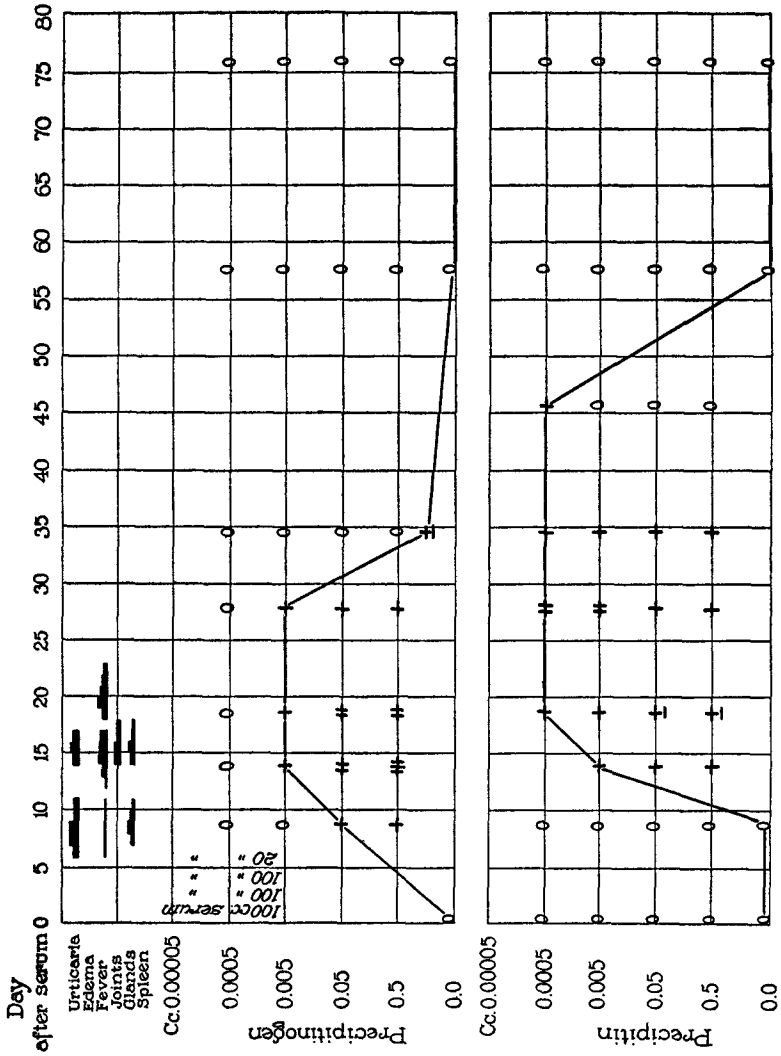
the termination of the serum reaction to be marked by the disappearance of these symptoms, ten of the eleven patients in this group had serum disease lasting 7 days or longer. The other patient had an urticarial eruption lasting 6 days. (2) A relatively high titer of precipitin appearing first or rising to the crest of the curve near the time that symptoms are subsiding. In only one of the patients in this group was the precipitin titer at the height of the curve less than 1:2,000. (3) Disappearance of precipitinogen from the circulation near the termination of the symptoms of serum disease. The usual form of curve for precipitinogen was a steady persistence at a high level until the precipitin curve rose sharply to its high point; a steep decline in the precipitinogen curve then occurred. Efforts were repeatedly made to demonstrate the presence of the foreign serum in the urine, but these tests were, without exception, negative. It seems probable, therefore, that the circulating precipitinogen is not eliminated by the kidneys, at least not in a form demonstrable by specific precipitation.

The characteristic interrelations of the three factors under consideration—precipitin, precipitinogen, and symptoms—are shown in Text-figs. 1 to 4. In Text-fig. 1, it is seen that the serum reaction began on the 5th day and persisted until the 34th day, that precipitin first appeared in the circulation on the 12th day, and rose to the crest of the curve synchronous with the disappearance of horse serum from the circulation and near the time that the symptoms subsided. Text-figs. 2 and 3 show very similar relations between precipitin, precipitinogen, and symptoms. Text-fig. 4 also conforms to this type but is of special interest because this patient had twice previously been treated with horse serum; 7 years before, he had been given diphtheria antitoxin without any reaction, and 2 years previously, he had meningococcus meningitis and was given antimeningococcus serum intraspinally. About 2 weeks later, he developed a severe attack of urticaria and his eyes were swollen. When we first saw him, he gave a positive intracutaneous reaction to horse serum and an attempt to desensitize him by the Besredka method of fractional doses was attempted. After a total of 34 cc. had been given, he developed an immediate reaction consisting of erythema and urticaria. This persisted for 24 hours, then 2 days later, that is on



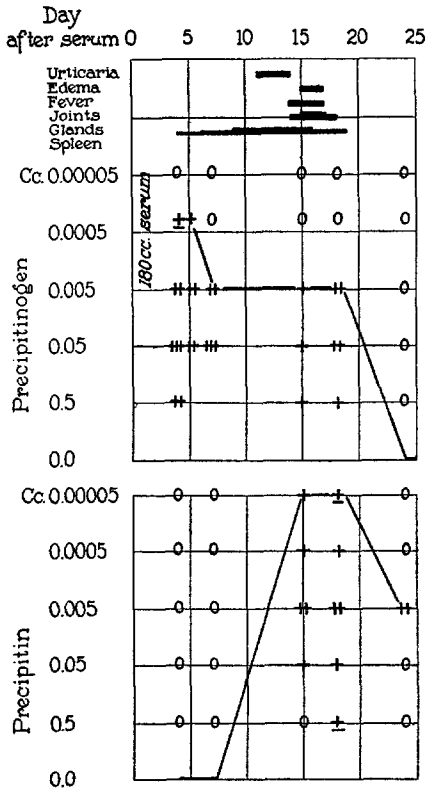
TEXT-FIG. 1. Patient 17 (Table I). Lobar pneumonia, Type I. Serum injections were made on the first 4 days.

In all the text-figures the abscissæ represent the time in days following the first administration of serum. The ordinates represent the dilutions of antigen used in the tests for precipitin and precipitinogen. At the top of the figure the symptoms of the serum reaction are shown in black. The results of the precipitin reactions in the various dilutions employed are shown by + and 0 signs. In the text-figures in which the amounts of serum for the different injections are shown it has sometimes been necessary, in order to make the lettering large enough to be legible, to include a larger number of days for serum injections than was actually involved. When this has been done the actual number of days on which serum was given, is indicated in the legend of the figure.



TEXT-FIG. 2. Patient 6 (Table I). Lobar pneumonia Type I. Serum injections were made on the first 2 days.

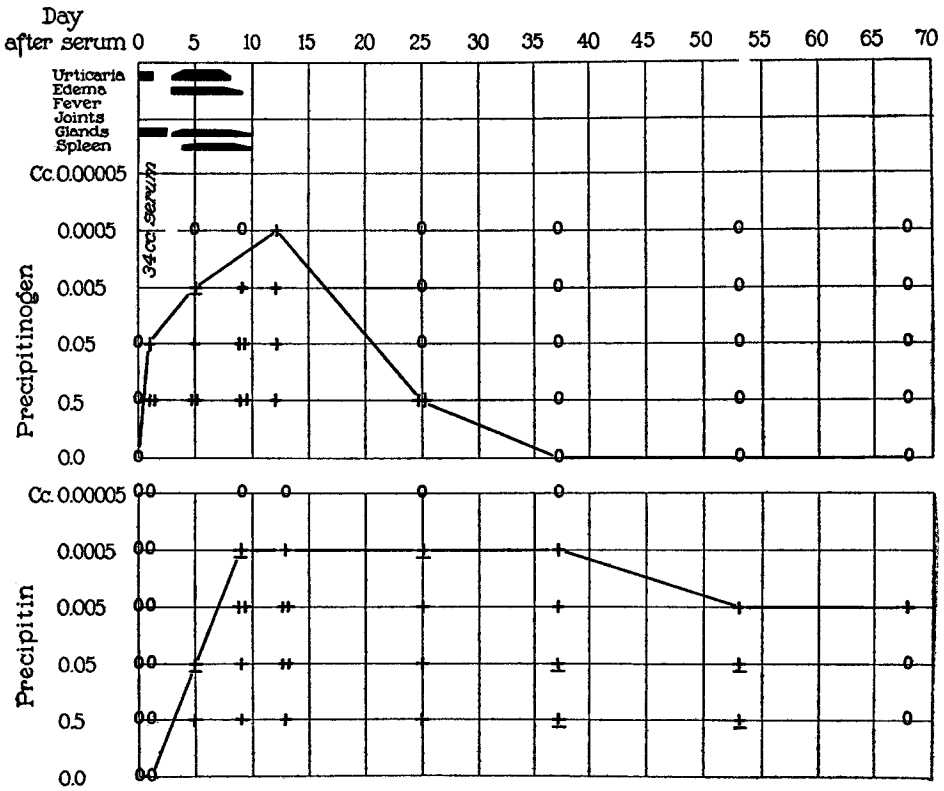
the 3rd day following the serum injections, an accelerated reaction developed, characterized by a morbilliform, urticarial, and purpuric eruption, edema, and enlargement of the lymph nodes and spleen. The particularly interesting feature was the accelerated appearance of precipitin, parallel with the accelerated symptomatic reaction. It is readily seer in Text-fig. 4 that the interrelations between pre-



TEXT-FIG. 3. Patient 14 (Table I). Lobar pneumonia Type I.

cipitin, precipitinogen, and symptoms were essentially the same in this reinjected patient, who showed immediate and accelerated reactions, as in patients receiving serum for the first time. The chief difference appears to be a shortening of the incubation period both for symptoms and for antibody formation. This is comparable to the early appearance of antibodies upon reinjection of rabbits pre-

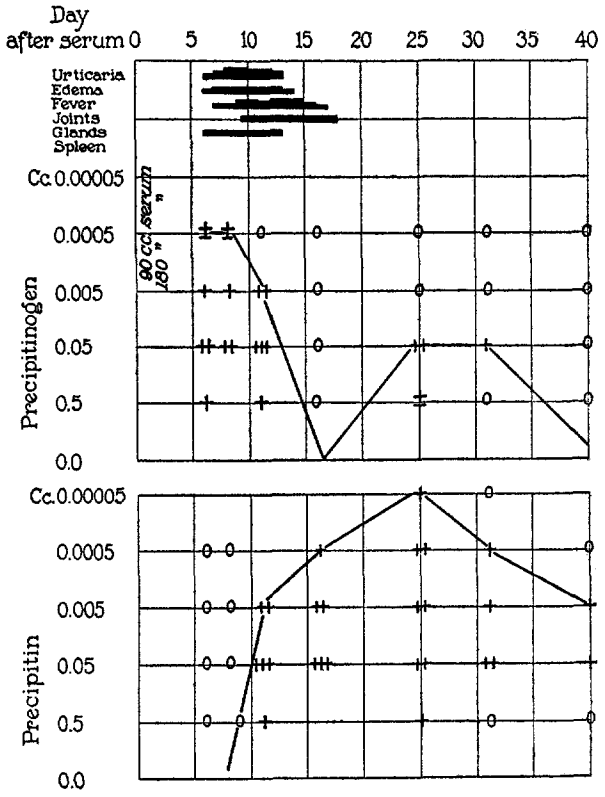
viously sensitized, but without antibody free in the circulation at the time of reinjection. The other seven patients in this group show similar relations between precipitin, precipitinogen, and symptoms. No two are exactly alike, nor would one, from analogy with antibody formation in animals, expect them to be so. The duration of the symptoms, the rapidity with which the precipitinogen disappears,



TEXT-FIG. 4. Patient 12 (Table I). Lobar pneumonia.

and the height of the precipitin curve show considerable variations, but in an unmistakable way, the characteristics of this group are manifest in all of the eleven patients. However, there are two patients in this group presenting a phenomenon demanding further comment. In Text-fig. 5, it is seen that following the rise of precipitin on the 11th day, the precipitinogen completely disappears from

the circulation, but appears again later, and after 9 days gives a positive reaction in dilutions up to 1:200. In following precipitin and precipitinogen in the circulation of rabbits, we have several times encountered a similar phenomenon. It seems as if under certain conditions the union of precipitin and precipitinogen were

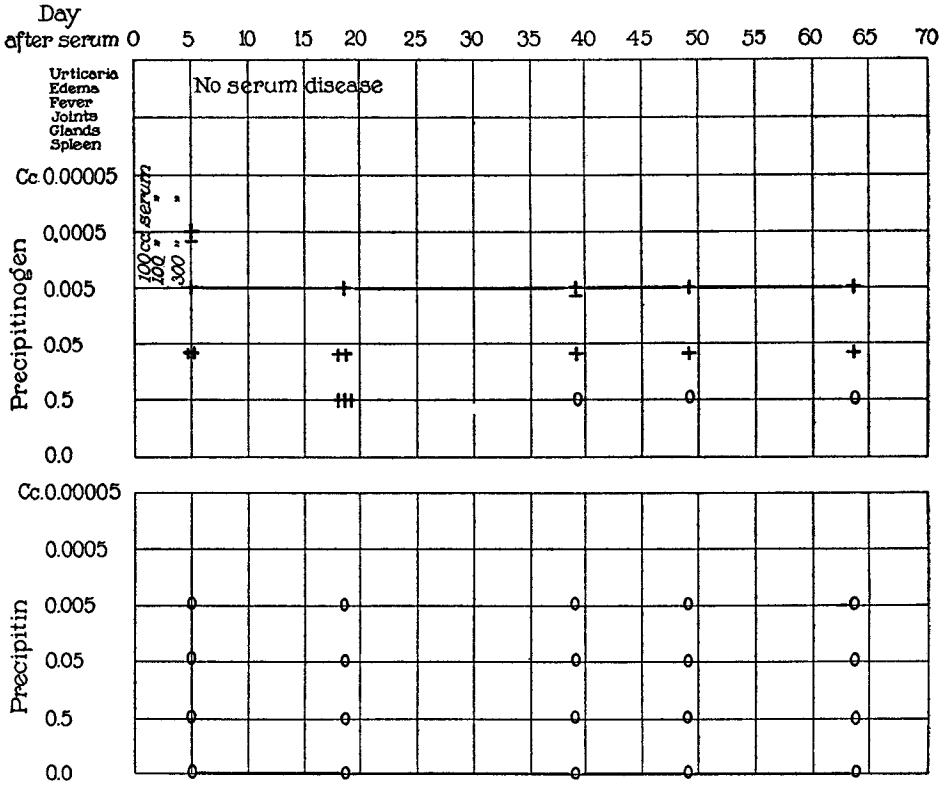


TEXT-FIG. 5. Patient 11 (Table I). Lobar pneumonia Type I. Serum injections were made on the first 2 days.

unstable and after being bound for a time by precipitin, the precipitinogen is subsequently released and circulates freely in the blood stream. We have no adequate explanation to offer for this reappearance of precipitinogen.

Group 2.—The patients in this group, four in number, form an interesting contrast with those just described. Instead of a severe

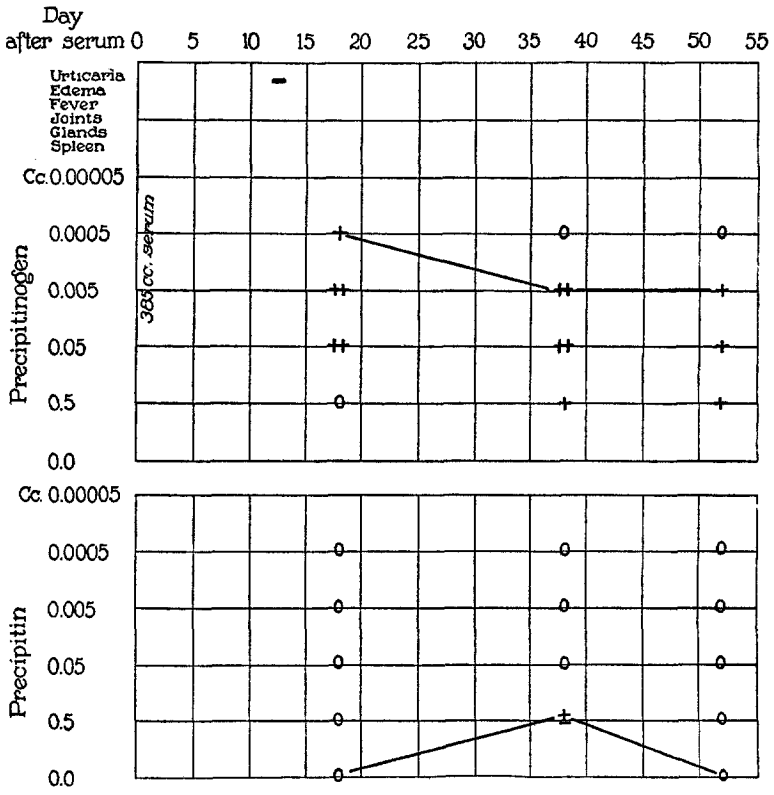
serum reaction, there is none at all or only very mild symptoms; instead of a high titer of precipitin, none is demonstrable in the circulation, or perhaps traces are present for a short period; instead of an early disappearance of precipitinogen, in each of the four patients of this type, there was a positive reaction for precipitinogen as long as the patient could be kept under observation—from 52 to 67 days.



TEXT-FIG. 6. Patient 13 (Table I). Lobar pneumonia Type I. Serum injections were made on the 1st, 2nd, and 4th days.

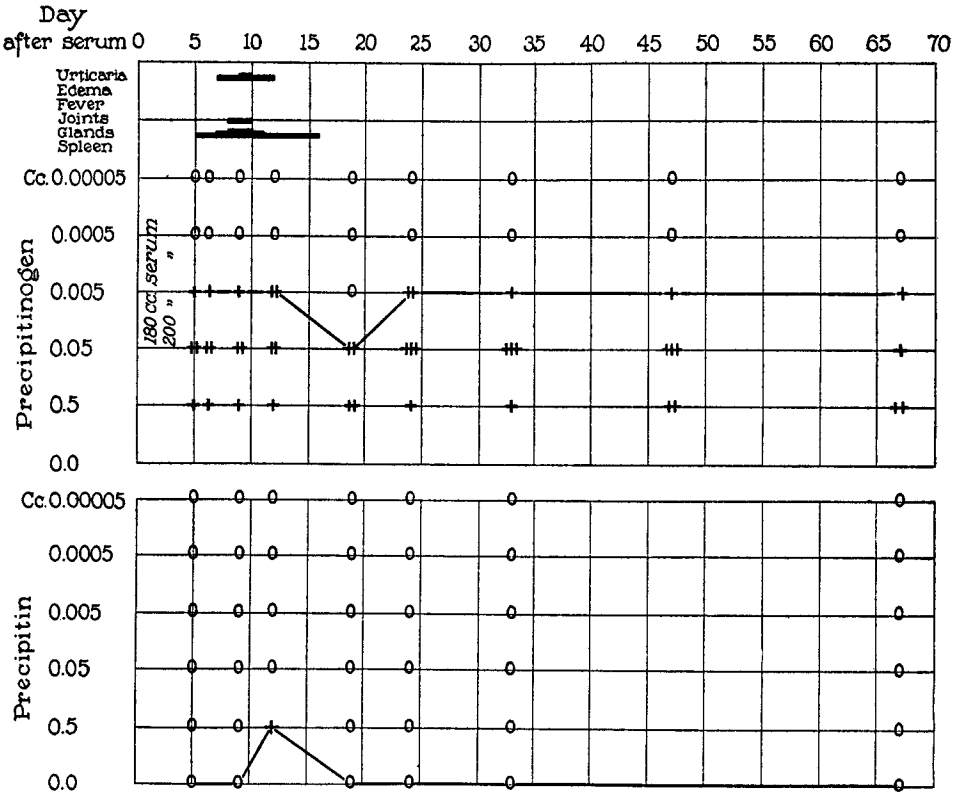
Text-figs. 6 to 8 illustrate the relations between precipitin, precipitinogen, and symptoms which characterize this group. In Text-fig. 6, it is seen that this patient, although given 500 cc. of serum intravenously, had no symptoms of serum disease, that precipitin did not appear in the circulation, and that horse serum was still present in the circulation 63 days after the first administration of serum. Text-

figs. 7 and 8 show relations of the same type between the three factors under consideration, although in each of these patients, there was a mild serum reaction and a transient appearance of precipitin in the lowest dilution.



TEXT-FIG. 7. Patient 16 (Table I). Lobar pneumonia Type I.

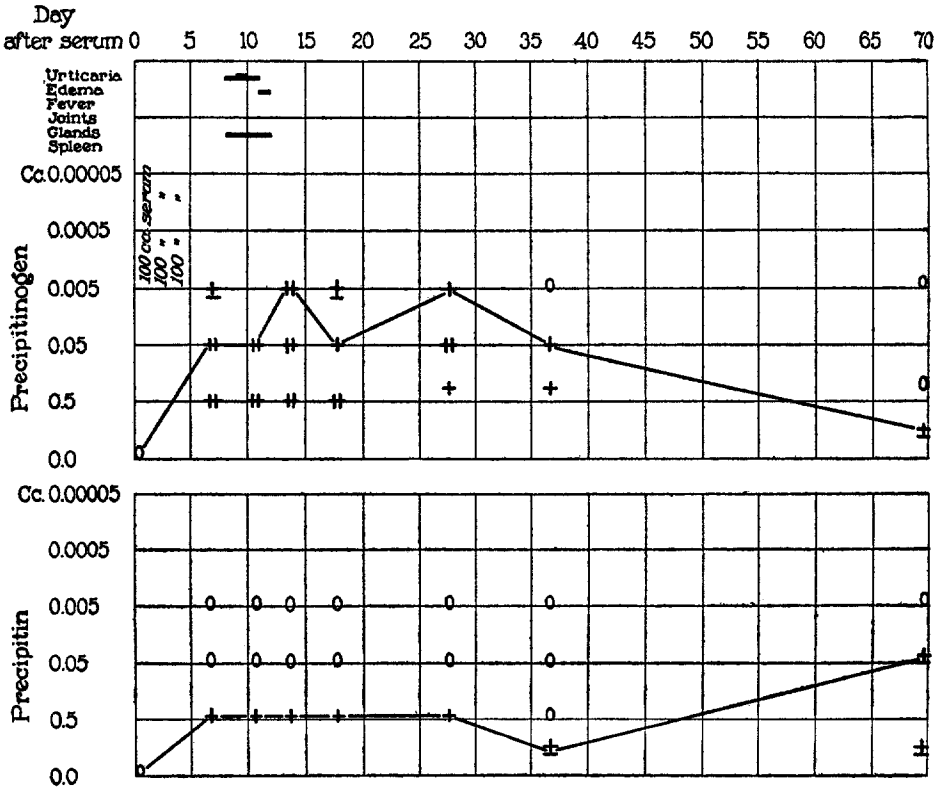
Group 3.—The four patients remaining do not fall definitely into either of the two groups, the characteristics of which have been described. Three of these four patients, however, had mild serum disease and transient or scanty precipitin in the circulation. One of these three is quite clearly a type intermediate between the two groups already described. Text-fig. 9 shows that this patient had a mild serum reaction, a very low titer of precipitin in the circulation, and a curve for precipitinogen which fell slowly, being intermediate



TEXT-FIG. 8. Patient 15 (Table I). Lobar pneumonia Type I. Serum injections were made on the first 2 days.

between the steep decline characteristic of the severe serum disease group and the steady persistence of the precipitinogen characteristic of the group which had no serum disease, or only very slight symptoms. One other patient was perhaps of this same type, but he was lost to observation before the curves could be completed. He had mild serum disease, transient appearance of precipitin, and a precipitinogen curve which showed a decline after the temporary appearance of precipitin, but did not reach the base-line. Owing to the fact that observation of this patient could not be continued, it was not possible to demonstrate that precipitin reappeared in the circulation or to determine when precipitinogen disappeared.

The third patient in this group of four which showed atypical curves was a patient with meningococcus meningitis who was given 95 cc. of antimeningococcus serum intraspinally. She had a very mild serum disease, a low titer of precipitin, and a curve for precipitinogen resembling that found in the patients with severe serum



TEXT-FIG. 9. Patient 5 (Table I). Lobar pneumonia Type I; pregnancy. Serum injections were made on the first 3 days.

disease. It is possible that when serum is administered intraspinally, some additional mechanism comes into play for the disposal of the foreign serum and, therefore, that the interrelations of symptoms, precipitin, and precipitinogen differ somewhat from those found in patients to whom the serum is administered intravenously. Recent

observations by Alexander[4] show that subdural and intravenous injections of the same quantities of foreign serum produce in rabbits distinctly different precipitin curves.

There remains one patient to be considered. This individual was given 630 cc. of antipneumococcus serum intravenously. He had a severe serum reaction; a high titer of precipitin appeared in the circulation synchronous with the cessation of the serum disease, but the precipitinogen, although falling somewhat at the time that precipitin rose to a high concentration, never disappeared entirely from the circulation during the 74 days that he was under observation. This is at variance with the relations which we have found to hold true in the other eighteen patients. There is, however, one consideration which might explain the discrepancy. This patient was one of the first studied, and at this time we had not realized the importance in precipitinogen determination of testing the anti-horse rabbit serum for traces of antigen used in immunizing the rabbit. It is possible, therefore, to explain the discrepancy on the assumption that the precipitating serum used contained traces of antigen and that the precipitin in the patient's serum gave a precipitate with the antigen still present in the anti-horse rabbit serum. This, of course, would make it appear that there was precipitinogen in the circulation of the patient, when it had, perhaps, entirely disappeared. It cannot be proved that this explanation is correct, but it seems plausible.

Summarizing then the results on nineteen patients, we find that eleven of them are distinctly of one type. These are the good precipitin formers who have severe serum disease and get rid of precipitinogen soon after the symptoms have subsided. The second group, contrasting sharply with these, consists of four patients. They are poor precipitin formers, have little or no serum disease, and retain the precipitinogen in the circulation for many weeks and perhaps months. Three of the remaining four patients are more or less distinctly intermediate forms, and the single patient in whom the results appear completely at variance with the observations on the other eighteen may perhaps be explained by a technical error.

[4] Alexander, H. L., *J. Exp. Med.*, 1921, xxxiii, 471.

DISCUSSION.

It has long been known that individuals show wide variations in their reaction to the administration of foreign serum. At one end of the scale is the individual who is naturally hypersensitive to horse serum. He is often a horse asthmatic with an inherited tendency to hypersensitiveness. He is exquisitely hypersensitive, reacting immediately with violent symptoms to even minute quantities of horse serum. Reactions of this type are sometimes fatal. At the other end of the scale is the individual who does not show a serum reaction after the intravenous administration of even several hundred cubic centimeters of serum. There is clearly wide variation in the response to foreign serum among individuals who at the time that serum is first administered are not demonstrably hypersensitive. Some have severe serum disease, some have only mild symptoms of short duration, and some have no symptoms whatever. That such variations, when large amounts of serum are given, are not dependent upon the amount of serum administered is evident from the results in the present series of patients. The average amount of serum given to the four patients in the group showing no serum disease, or only very mild symptoms was 460 cc.; the average amount given to the patients who had severe serum reactions was 270 cc. When smaller amounts of serum are used, as in diphtheria immunization, and the injections are made subcutaneously or intramuscularly, there appears to be a relation between the incidence of serum reactions and the amount used.[5] With the larger doses, the incidence of reactions is higher, but when amounts over 100 cc. are given, the variations in the intensity and duration of the reaction lead to the conclusion that there is an intrinsic difference between the susceptible and the insusceptible individuals.

What we have found to be characteristic of the susceptible group of patients as far as precipitin, precipitinogen, and symptoms are concerned corroborates the observations of Longcope and Rackemann[1] on the relation of circulating precipitin to the cessation of symptoms, and lends further support to the conception of serum dis-

[5] Daut, M., *Jahrb. Kinderheilk.*, 1897, xliv, 289. Sturtevant, M., *Arch. Int. Med.*, 1916, xvii, 176. Weaver, G. H., *Arch. Int. Med.*, 1909, iii, 485.

ease as an antigen-antibody reaction. Efforts to discover the mechanism of antigen-antibody reactions have resulted in the development of two hypotheses which differ as to the site in which the reaction occurs. One school localizes the reaction within the circulation and holds to the view that the reaction is dependent upon an intravascular union of antigen and antibody. The other school believes that this union takes place chiefly within the tissue cells. No attempt will be made to discuss the voluminous and controversial literature on this question, but it suffices to say that, at present, a preponderance of competent opinion supports the cellular hypothesis. With the facts at present available, the most plausible explanation of the mechanism of these reactions[6] is, therefore, that the foreign serum unites with the tissue cells; that as a result of such union, antibodies are produced; that the intracellular union of antigen and antibody causes the symptoms; and that after a time, the antibody is produced in excess and ceases then to be exclusively intracellular. At this time it becomes demonstrable in the circulation. For a time thereafter, there are both antigen and antibody free in the circulation. If large amounts of antibody reach the circulation, the antigen soon disappears. This disappearance of antigen does not necessarily imply an intravascular union of antigen with antibody, although such a mechanism might account for it. It is also possible that at this time, with greater amounts of intracellular antibody available, the tissue cells appropriate the antigen with greater avidity. All that can be stated at present is that during this period when precipitin is abundant, the foreign serum disappears.

The varying degrees of intensity of reaction by susceptible individuals should therefore be thought of either as varying degrees of susceptibility to a toxic product of intracellular antigen-antibody reaction, or as varying degrees of ability to form antibodies. The latter hypothesis seems to accord better with the facts, because, in general, it is the good precipitin formers who have severe serum reactions. The parallelism is not exact, nor would one expect it to be so, unless there were proof that the precipitin in the circulation is exactly proportional to the intracellular precipitin. And there is no

[6] Weil, R., *J. Immunol.*, 1916–17, ii, 399.

proof of this. A possible explanation of such varying degrees of efficiency with which different individuals form antibodies is discussed below.

In the group of patients found to be insusceptible to serum reactions, in whom little or no precipitin is found, and in whose circulation the foreign serum is present for a long period, there clearly is something which acts as a protective mechanism. Three possibilities perhaps offer plausible explanations for the basis of this failure to produce precipitins and the persistence of the precipitinogen in the circulation. (1) In these individuals there may be something in the circulation which stands as a barrier between the foreign protein in the blood stream and the interior of the cells, preventing the union of foreign protein and tissue cell, and thus interfering with an essential phase of antibody production. (2) The tissue cells of these individuals may be impermeable to the foreign serum. If either one of these conditions existed, little or none of the foreign serum would penetrate the cells, and hence little or no antibody would be formed and serum disease would be mild or absent. (3) In terms of Ehrlich's side-chain theory, the difference between the susceptible and the insusceptible individuals might consist in a deficiency or absence of haptophore groups in the tissue cells of the latter. Under any circumstances, with failure of the cells to take up the foreign protein, it might persist in the circulation until slowly disposed of by some other mechanism.

It is interesting in this connection to recall some observations made years ago by Metchnikoff.[7] In studying the mechanism of natural immunity, he found that a number of species were insusceptible to certain bacterial toxins which were extremely poisonous for other species. Spiders and scorpions were found to be unaffected by tetanus toxin. Nor could antitoxin be demonstrated in the blood of these animals, so the natural immunity could not be ascribed to antitoxic properties of the body fluids. In these animals, however, the injected toxin soon disappeared from the circulation. The immunity of fowls to tetanus toxin was known even before Metchnikoff's studies. The fowl also tolerates large doses of tetanus toxin but can nevertheless be tetanized if it is previously weakened by exposure to cold or if sufficiently large doses are used. That this immunity is not due to the presence or production of antitoxin was shown by Vaillard[8] who

[7] Metchnikoff, E., Immunity in infective diseases, translated by Binnie, F. G., Cambridge, 1905.

[8] Vaillard, *Compt. rend. Soc. biol.*, 1891, xliii, 462.

demonstrated that the serum contains no antibody. Moreover, von Behring[9] showed that fowls are highly susceptible if the toxin is injected directly into the brain. Metchnikoff also studied green lizards, marsh turtles, and the larvæ of the rhinoceros beetle (*Oryctes nasicornis*). These animals tolerate, without symptoms, enormous doses of tetanus toxin and not only do not form any antitoxin, but retain the toxin in the circulation for a long period of time, even months.

Phenomena of this type in lower animals suggested to both Metchnikoff[7] and von Behring[10] the possible relation of cell permeability to natural immunity. Our observations on serum disease in man are strikingly analogous to those of Metchnikoff on turtles, lizards, and beetles and suggest anew a factor which may be important in natural immunity.

Investigators in the field of natural immunity have been occupied chiefly in studying different degrees of susceptibility characterizing races or species. On epidemiological grounds alone, it is clear, however, that within any given species or race, there are individual variations in susceptibility to infection for which no adequate explanation exists. From this standpoint, therefore, the demonstration of the differences which we have found to exist between susceptible and insusceptible individuals of the same race may prove to be significant.

SUMMARY.

1. Studies on nineteen patients to whom foreign serum had been administered for therapeutic purposes are reported. Analysis of the results obtained by following the precipitin and precipitinogen in the circulation and comparing these factors with the time of appearance, intensity, and duration of the symptoms shows that the nineteen patients fall into three groups.

2. The first group includes eleven patients. These were good precipitin formers, they had severe serum disease, and the precipitinogen disappeared from the circulation near the time that the symptoms subsided.

[9] von Behring, E., in Eulenberg, A., and Samuel, Lehrbuch der allgemeinen Therapie, Berlin and Vienna, 1899, iii, 992.

[10] von Behring, E., in Eulenberg, A., Encyclopädische Jahrbücher der gesammten Heilkunde, Vienna and Berlin, 1900, ix, 203.

3. The second group includes four patients who had little or no serum disease, in whose circulation little or no precipitin was demonstrable, and in whom the precipitinogen persisted in the circulation as long as the patients could be kept under observation—from 52 to 67 days.

4. The remaining four patients form a more or less distinctly intermediate group.

5. The results lend further support to the conception of serum disease as an antigen-antibody reaction.

6. The possibility that our results indicate a factor which may be important in the mechanism of natural immunity is discussed.

We wish to thank Miss Emily Frühbauer for technical assistance.